REMARKS

Applicants have carefully studied the Office Action mailed on July 8, 2003, which issued in connection with the above-identified application. The present response is intended to be fully responsive to all points of rejection raised by the Examiner and is believed to place the claims in condition for allowance. Favorable reconsideration and allowance of the present claims are respectfully requested.

Applicants gratefully acknowledge the courtesy shown by the Examiner and the Examiner's Supervisor Anthony Caputa in providing recommendations for response to the present Office Action in an in-person interview with Dr. Paul Fehlner on October 23, 2003. The Examiner's Interview Summary is attached herein as Exhibit A.

Pending Claims

Claims 2, 3, 11-16, 27-29, 48-51, and 54-62 were pending and at issue in the application. Claims 55, 27-29 and 58-59 have been rejected under 35 U.S.C. §112, second paragraph, as being indefinite. Claims 2-3, 11-15, 27-28, 48-51, and 54-56 have been rejected under 35 U.S.C. §102(e) and/or under 35 U.S.C. §102(b) and/or under 35 U.S.C. §102(a) as being anticipated by prior art. Claims 15-16, 27-29, 55-57, and 60 have been rejected under 35 U.S.C. §103(a) as being obvious over prior art.

Claims 27-28 and 55 have been canceled without prejudice or disclaimer. Claims 58 and 59 have been amended to correct formal defects. Claims 11-13 and 15 have been amended to correct dependency (with claims 13 and 15 re-written in dependent format). Claim 29 has been re-written in independent format. Claims 2, 48-49, 51, and 56 have been amended and new claims 63-65 have been added to more particularly point out and distinctly claim the invention. Specifically, claim 51 has been amended to further clarify the limitation in the preamble by adding the phrase "detecting inactivation of a *CASP8* gene expression in a cell from a subject,

wherein said inactivation of a *CASP8* gene expression in the cell is indicative of the presence of a cancer". Support for this recitation can be found, for example, at page 2, lines 27-28; page 3, lines 4-9; page 7, lines 11-28; page 28, lines 16-20; page 51, lines 13-16, and page 60, line 20 - page 61, line 7 of the specification and in the original claim 10. Claim 51 has been further amended to delete the term "prognosis", which is recited in the newly added claim 63. The newly added independent claim 63 is directed to a method for prognosis of a cancer comprising detecting inactivation of a *CASP8* gene expression and finds support, for example, at page 3, lines 4-9; page 28, lines 16-20; page 51, lines 13-16, and page 60, line 20 - page 61, line 7 of the specification and in the original claim 10. Newly added dependent claims 64 and 65 are directed to a method for detecting the absence of a *CASP8* mRNA and find support, for example, at page 30, lines 20-24 of the present specification. Claims 2, 48-49 and 56 have been amended to recite "detecting inactivation of a *CASP8* gene expression in a primary cancer cell". Support for this newly introduced limitation can be found, for example, at page 31, lines 24-26; page 54, line 25 - page 56, line 34, and Figures 7A-C. No new subject matter has been added as a result of these amendments, no new search is required, and no new issues are raised. Upon entry of the above-identified amendments, claims 2-3, 11-16, 29, 48-51, 54, and 56-65 will be pending.

35 U.S.C. § 112, Second Paragraph Rejections

In the Action, claims 55, 27-29 and 58-59 stand rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Specifically, the Examiner contends that while these claims are directed to kits, the term "assay" in claim 55 defines a method and not a product.

As claims 27-28 and 55 have been canceled, the rejection of these claims is rendered moot. Claim 29 has been re-written in independent format to recite a kit for detecting inactivation of a *CASP8* gene expression, comprising oligonucleotide primers for amplification

of at least a part of the 5' untranslated region of *CASP8* genomic DNA, wherein said primers are used in a methylation polymerase chain reaction (PCR) assay. Claims 58-59, which depend from claim 29, have been amended to replace the term "assay" with the term "kit". Applicants respectfully note that claims 29 and 58-59 as amended are directed to a product (kit) and not to a method. Moreover, these claims recite (i) specific components of the kit (*i.e.*, oligonucleotide primers used in a methylation polymerase chain reaction (PCR) assay) and (ii) a specific part of the genome (*i.e.*, a part of the 5' untranslated region of *CASP8* genomic DNA), which is used for amplification using said components.

In light of the above-presented amendments and arguments, the rejection under 35 USC §112, second paragraph, is believed to be overcome and withdrawal of such is kindly requested.

35 U.S.C. § 102 and 103 Rejections

In the Office Action, the Examiner has maintained the rejection of claims 2-3, 27-28, 48-50, and 54-56 under 35 U.S.C. §102(e) as being anticipated by U.S. Patent No. 6,172,190 to Hunter *et al.* The Examiner contends that Hunter *et al.* patent teaches methods for detecting the absence of caspase-8h or caspase-8i proteins (using immunoassay), detection of caspase-8h or caspase-8i mRNA, and detection of caspase-8h or caspase-8i methylation (using RNase protection assay). The Examiner further contends that Hunter *et al.* patent teaches PCR primers for use in PCR assays.

The Examiner has also rejected claims 2-3, 11-15, 27-28, 48-51, and 54-56 under 35 U.S.C. §102(b) as being anticipated by PCT Application No. WO 97/46662 by Dixit *et al.* The Examiner contends that, similarly to Hunter *et al.* patent, Dixit *et al.* application teaches methods for detecting the absence of caspase-8 (ICE LAP-7/FLICE) protein, detecting the absence of caspase-8 mRNA, and detecting methylation of caspase-8 genomic DNA (by RNase protection assay) as well as PCR primers for use in PCR assays.

Claims 15-16, 27-29, 55-57, and 60 have been also rejected under 35 U.S.C. §103(a) as being obvious over Dixit *et al.* application in view of the two Herman *et al.* references cited in the previous Office Actions (Proc. Natl. Acad. Sci. USA, 93: 9821-9826, 1996 and *ibid.* 91: 9700-9704, 1994). With respect to Herman *et al.* references, the Examiner maintains that it would be obvious to use the methylation-specific PCR detection of gene methylation taught by Herman *et al.* in the detection methods of Dixit *et al.*

In the Action, claims 27-28 and 55 stand further rejected under 35 U.S.C. §102(b) as being anticipated by PCT Application No. WO 97/03998 by Wallach *et al.* or by PCT Application No. WO 97/35020 by Alnemri *et al.* The Examiner contends that these applications disclose oligonucleotide primers for PCR detection of caspase-8[1] and therefore teach kits that are the same as claimed.

Claims 2-3, 11-14, 48, and 51 have been also rejected under 35 U.S.C. §102(b) as being anticipated by Scaffidi *et al.* (J. Biol. Chem., 272: 26953-8, 1997) or under 35 U.S.C. §102(a) as being anticipated by Juo *et al.* (Curr. Biol., 8: 1001-8, 1998). The Examiner contends that both articles teach the detection of the absence of the caspase-8 protein.

As claims 27-28 and 55 have been canceled, the rejection of these claims is rendered moot. With respect to the remaining claims, the rejection is respectfully traversed.

Claims 2-3, 48-50, 54, 56-57, and 60-62 as amended recite detecting inactivation of a *CASP8* gene expression <u>in a primary cancer cell</u>. As acknowledged by the Examiner during the interview, none of the references cited in the Office Action disclose or suggest detecting inactivation of a *CASP8* gene expression in a primary cancer cell. In fact, it is widely recognized in the field that the present inventor and co-workers were the first to demonstrate and report that

[1] The Examiner states that these oligonucleotide primers are used for PCR detection of caspase-8 <u>proteins</u> (see pages 5 and 6 of the Office Action). Applicants respectfully note that PCR only allows detection of nucleic acids (DNA and RNA) and cannot be used to detect proteins. Correction is respectfully requested.

(i) suppression of CASP8 expression correlates with tumors and (ii) such suppression of CASP8 expression is frequently achieved through methylation of the 5' untranslated region of *CASP8* genomic DNA (see, *e.g.*, reviews by Juin and Evan in Nature Med., 6: 498-500, 2000 and by Borriello *et al.* in Haematologica, 87: 196-214, 2002 (especially page 206), attached as Exhibits B and C, respectively). Accordingly, claims 2-3, 48-50, 54, 56-57, and 60-62 are not anticipated by or obvious over the cited art.

With respect to claims 56-57 and 60-62 directed to a method for detecting inactivation of *CASP8* gene expression, comprising detecting methylation of *CASP8* genomic DNA, applicants also note that none of the references cited by the Examiner disclose or suggest detecting methylation of *CASP8* genomic DNA. In fact, none of these references disclose or suggest that *CASP8* genomic DNA is methylated or that such a methylation can cause inactivation of the *CASP8* gene expression. Indeed, the Examiner acknowledges at page 8 of the Office Action (lines 1-2) that "Dixit fails to teach or suggest method for detection of methylation in the 5'-untranslated region of caspase-8 genomic DNA."

Applicants further respectfully submit that the RNase protection assays disclosed in the cited references do not constitute a method for detecting methylation of genomic DNA, but rather a general method for detecting the level of specific mRNA. In this respect, the RNase protection assay is similar to a Northern analysis or RT-PCR.

Methylation of genomic DNA involves addition of methyl groups to cytosines in CpG dinucleotides of genomic DNA, which, when occurring in the 5' untranslated region of a gene, may inhibit the expression of this gene, *e.g.*, by affecting assembly of transcriptional complexes. See, *e.g.*, recent reviews by Leone *et al.* (Haematologica, 87: 1324-1341, 2002) and Singal and Ginder (Blood, 93: 4059-4070, 1999), attached as Exhibits D and E, respectively. Unlike other methods of regulating gene expression, methylation involves a covalent chemical modification of genomic DNA. Accordingly, the substrate for detecting methylation of genomic DNA can only

be genomic DNA. As described in the review by Singal and Ginder (see pages 4060-4061), the most frequently used assays for detecting methylation of genomic DNA are digestion with methylation-sensitive restriction enzymes followed by Southern hybridization or PCR, Maxam and Gilbert sequencing, and a combination of bisulfite-induced oxidative deamination and PCR (the latter method is disclosed and claimed in the present application).

In contrast to assays for detecting methylation of genomic DNA which use genomic DNA as a substrate, the substrate in RNase protection assays is only RNA and not genomic DNA. RNase protection assay is a method for detection and quantitation of RNA species (usually mRNA) as well as for mapping mRNA termini and intron/exon junctions. RNase protection assay employs a labeled RNA probe that is complementary to part of the target RNA to be analyzed (see the description of the method provided in the on-line catalog of Ambion, Inc., attached as Exhibit F). After hybridization of the labeled probe and sample RNA, the mixture is treated with RNase to degrade unhybridized probe. Labeled probe that is hybridized to complementary RNA from the sample is protected from RNase digestion and can be visualized upon electrophoretic separation.

The difference between RNase protection assays and methods for detecting methylation of genomic DNA is well exemplified in the two references, Matsumura *et al.*, Clin. Cancer Res., 7: 594-599, 2001 and Dieguez *et al.*, Mol. Gen. Genet., 253: 581-588, 1997, attached as Exhibits G and H, respectively. In both references, RNase protection assays are used solely to detect the expression of mRNAs (see, *e.g.*, page 596, bottom of right column in Matsumura *et al.* and page 585, bottom of right column in Dieguez *et al.*), while to detect methylation of genomic DNA both references use digestion with methylation-sensitive restriction enzymes (see, *e.g.*, page 595, bottom of left column in Matsumura *et al.* and page 582, last complete paragraph in the right column in Dieguez *et al.*).

It follows that, in contrast to the Examiner's assertion, disclosure of RNase protection assay in the cited references cannot be construed as the disclosure of the assays capable of assessing methylation of genomic DNA. Accordingly, claims 56-57 and 60-62 are not anticipated by or obvious over the cited art.

With respect to claims 51 and 11-15 directed to methods for diagnosis or prognosis of a cancer comprising detecting inactivation of a caspase-8 gene expression, applicants respectfully disagree with the Examiner's position expressed in the sentence bridging pages 4 and 5 of the Office Action that these claims "fail to relate the method steps to the purpose stated in the preamble, and therefore are interpreted as reading on methods that comprise steps of detecting the absence of expression of CASP8..." Applicants respectfully note that, according to the present law, preamble terms are limitations, and give life to the claim, when they are "necessary to give meaning" to the claims and to "properly define" the invention, or are "integral to the claim itself," as by distinguishing the prior art and providing antecedent basis for terms in the body of the claim. *Gerber Garment Technology, Inc. v. Lectra Systems, Inc.*, 916 F.2d 683, 689 (Fed. Cir. 1990). As in *Kropa v. Robie*, 187 F.2d 150, 152 (CCPA 1951), a preamble "counts" when it is, "essential to point out the invention defined by the claim." The preamble is a claim limitation, and not just an introduction, when it cooperates with the rest of the claim, "so as to distinguish the claim ... over the prior art." *Id.* See also *Bristol-Myers Squibb Co. v. Ben Venue Laboratories, Inc.*, 246 F.3d 1368, 1373-1377 (Fed. Cir. 2001).

In light of the above law, applicants respectfully note that, in contrast to the Examiner's assertion, claims 51 and 11-15 are directed to methods for diagnosis or prognosis of a cancer comprising detecting inactivation of a caspase-8 gene expression and not to methods for detecting inactivation of a caspase-8 gene expression. To clarify this further, claim 51 has been amended to recite "detecting inactivation of a *CASP8* gene expression in a cell from a subject, wherein said inactivation of a *CASP8* gene expression in the cell is indicative of the presence of

a cancer" and to delete the term "prognosis", which is recited in the newly added claim 63. The newly added independent claim 63 is directed to a method for prognosis of a cancer comprising detecting inactivation of a *CASP8* gene expression, wherein said inactivation of a *CASP8* gene expression is indicative of the inefficiency of apoptosis induced by activated death receptors, chemotherapeutic drugs, or irradiation. As specified above and acknowledged by the Examiner during the interview, none of the references cited in the Office Action disclose or suggest that detecting inactivation of *CASP8* gene expression can be used for diagnosis or prognosis of a cancer. In fact, it is widely recognized in the field that the present inventor and co-workers were the first to demonstrate and report that (i) suppression of CASP8 expression correlates with aggressive tumors which are particularly resistant to induction of apoptosis and (ii) such suppression of CASP8 expression is frequently achieved through methylation of the 5' untranslated region of *CASP8* genomic DNA (see, *e.g.*, reviews by Juin and Evan in Nature Med., 6: 498-500, 2000 and by Borriello *et al.* in Haematologica, 87: 196-214, 2002 (especially page 206), attached as Exhibits B and C, respectively). Accordingly, claims 51 and 11-15 as well as new claims 63-65 are not anticipated by or obvious over the cited art.

With respect to obviousness rejection of claims 15, 16, 29, 56-57, and 60 over Dixit *et al.* application in view of the two Herman *et al.* references, applicants respectfully submit that Herman references do not cure any of the deficiencies of Dixit *et al.* or any of the other cited references. Although Herman references teach methylation (including methylation of a promoter region) as a mechanism for silencing of tumor-suppressor genes, they do not teach or suggest methylation of *CASP8* genomic DNA or any other gene modification leading to the lack of expression of CASP8 protein. As specified above, there is no evidence in any of the other cited references that *CASP8* genomic DNA is methylated nor that such a methylation can cause inactivation of the *CASP8* gene expression. Therefore, one skilled in the art would not be motivated to look for methylation or lack of expression of CASP8 based on the combination of

these references, *i.e.*, there is no motivation to combine Herman with the other references. In other words, the actual teachings of the references taken as a whole do not suggest the claimed invention, and the rejection requires impermissible hindsight reconstruction of various unconnected bits and pieces of the references to sustain itself. It is well settled however, that such hindsight reconstruction is an error (see the summary of law provided in response to the previous Office Action).

Applicants further note that the specific PCR primer sequences recited in the present claims 59 and 62 (*i.e.*, SEQ ID NOS: 29-34) allow the amplification of 5' untranslated region of *CASP8* genomic DNA (*i.e.*, SEQ ID NO: 1 or 2), which is not found within the sequences disclosed in the references cited in the Office Action. Accordingly, in contrast to the Examiner's assertion, the claims reciting oligonucleotide primers for use in PCR assays are not anticipated by or obvious over the cited art.

In summary, applicants respectfully submit that, even if taken together, the cited references do not disclose or suggest the methods and kits encompassed by the present claims. It follows that rejected claims as well as new claims 63-65 are not anticipated by or obvious over the cited art. Reconsideration and withdrawal of the anticipation and obviousness rejections is believed to be in order.

CONCLUSION

Applicants request entry of the foregoing amendments and remarks in the file history of this application. In view of the above amendments and remarks, it is respectfully submitted that claims 2-3, 11-16, 29, 48-51, 54, and 56-65 are now in condition for allowance and such action is earnestly solicited. If the Examiner believes that a telephone conversation would help advance the prosecution in this case, the Examiner is respectfully requested to call the undersigned agent

at (212) 527-7634. The Examiner is hereby authorized to charge any additional fees associated with this response to our Deposit Account No. 04-0100.

Respectfully submitted,

Dated: December 3, 2003

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